Summary

Saponin (II) (a mixture of polar oligosides), the major component of the leaf saponins of *Digitalis purpurea* L., was found to be enzymatically cleaved to yield solely the minor component, saponin (I) (a mixture of two tetraglycosides, desgalactotigonin and F-gitonin).

Saponin (I), subsequently the component two tetraglycosides, were conveniently prepared from the crude leaf saponins precipitated by cholesterol and more efficiently from the butanol extract of the leaves by the specific partial hydrolysis of saponin (II) with some commercial enzyme preparations.

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57. **Keishi Kotera**: The Conformational Studies of Vitamin B_1 Analogues by the Proton Magnetic Resonance Spectroscopy.

(*Osaka Research Laboratory, Tanabe Seiyaku Co., Ltd.*[*1])

Recently, Vitamin B_1 has been improved to provide maintainable efficiency, higher absorbability and inertness to Thiaminase. Vitamine B_1 analogues can be classified into two groups as regards the thiazole ring cyclizing or not, and the latter case can be divided into the symmetric disulfide type and other sulfide derivatives.

In the present paper, the chemical shifts and the temperature dependence of the proton magnetic resonance spectra were studied to obtain the conformational relation between pyrimidine ring and thiazole ring or N-formyl group.

Experimental

For the following various vitamin B_1, either recrystallized from the commercial reagents or synthesised by the usual method, the proton magnetic resonance (NMR) spectra were measured on Japan Electron Co. J.N.M-C60 spectrometer at 60 Mc. About 15% solutions of the samples, in $N/2$ HCl acidic deuterium oxide added a few drops of dioxane as internal standard, or in $CDCl_3$ with TMS, were prepared. The temperature of these sample was changed over the range from −2° to 80° by Japan Electron Co. JES-VT-2 Temperature Controller.

Samples :

Thiamine	[T]	O-Benzoylthiamine	[OBT]
Thiothiamine	[TT]	S-Benzoylthiamine monophosphate	[BTMP]
S-Methylthiamine	[MT]	Thiamine disulfide	[TDS]
Thiamine diphosphate	[TDP]	Thiamine disulfide monophosphate	[TDMP]
		O-Benzoylthiamine disulfide	[BTDS]

Results and Discussion

The proton chemical shifts of various vitamin B_1 are shown in Chart 1. Further the influence of substituted oxyethyl group and cyclization of thiazole ring are described in Table I.

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The magnetic anisotropy of substituents, as O-benzoyl and O-phosphoyl, given the same deshield effect for oxyethyl protons in the thiazole type and the disulfide type. The proton of pyrimidine ring or N-formyl was invariant by the substitution of phosphoyl group, but benzoyl group given the higher field of 0.2~0.3 p.p.m. Although, the oxyethyl methylene protones of BTMP were not affected by the existence of S-benzoyl group.

By the cyclization of thiazole ring, all protons undergone the lower field shift due to the ring current effect of thiazole ring.

The temperature dependence of the NMR spectra of BTDS in $CDCl_3$ was shown in Fig. 1. At the lower temperature, the broad quartet with the value of J≈12 c.p.s. and δ AB≈0.7 p.p.m. overlapped to the triplet at 5.66 τ ($-CH_2-O$), was assigned to the AB type of methylene attached to the pyrimidine ring and N-formyl group.

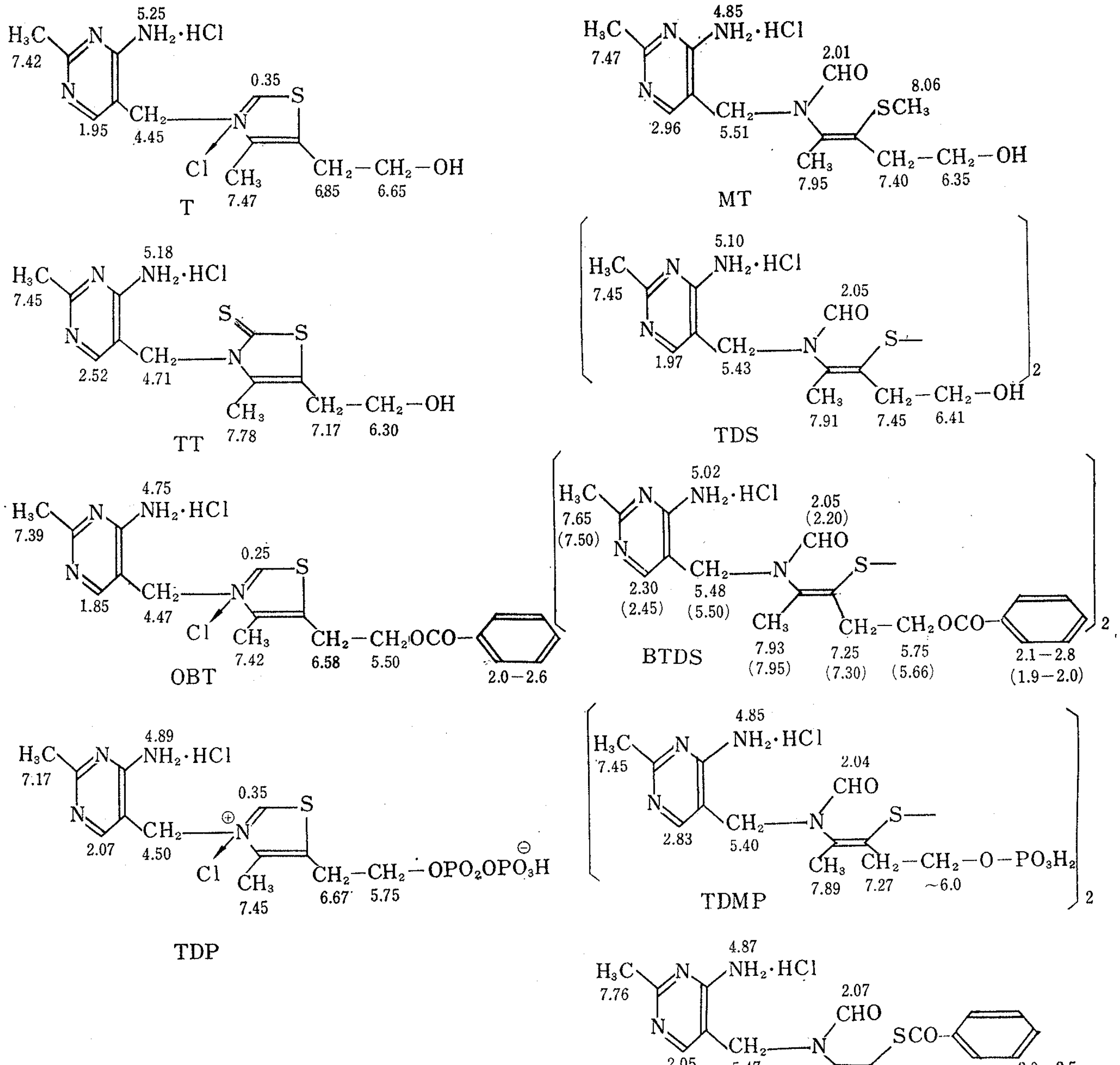

Chart 1.

The proton chemical shift in D_2O solutions denoted by the same scales as τ-value, where the internal standard dioxane appear at 6.30 p.p.m. Parenthesis of BTDS represents the values in $CDCl_3$ solution.

This quartet was changed to the extensive broad signal at room temperature. As the temperature increases, this broad signal was gradually narrowed, resulting in the singlet pattern with the same sharpness as internal standard TMS while the two methylene signals of benzoyloxyethyl group were also eventually changed into the normal triplet with the binominal intensity ratio. But the CH protons of pyrimidine ring and N-formyl group were almost independent of temperature.

Under the various tempertures, the methylene signal of BTDS in $CDCl_3$ solution can be represented by the factor $2\pi\tau\Delta\nu$, as pictured by Grunwald, *et al.*[1] From the equation given by Gutowsky and Holm,[2] the relation of $2\pi\tau\Delta\nu$ and $1/T$ are illustrated as a straight line of Fig. 2, in which a slope represents the energy barries *Ea*, about 9.6 kcal./mole, the torsional vibration factor *Ko* of 0.3×10^4 c.p.s.

In $N/2$ acidic deuterium oxide solution, similar spectral changes were observed as the difference in half width of the methylene signals (Table II). The temperature dependence were shown in the disulfide type thiamine as BTDS, TDS, and TDMP, and BTMP, whereas all the signals of parent thiamine were sharp and unaffected by a temperature, and the signal changes of TDP and OBT were also slight.

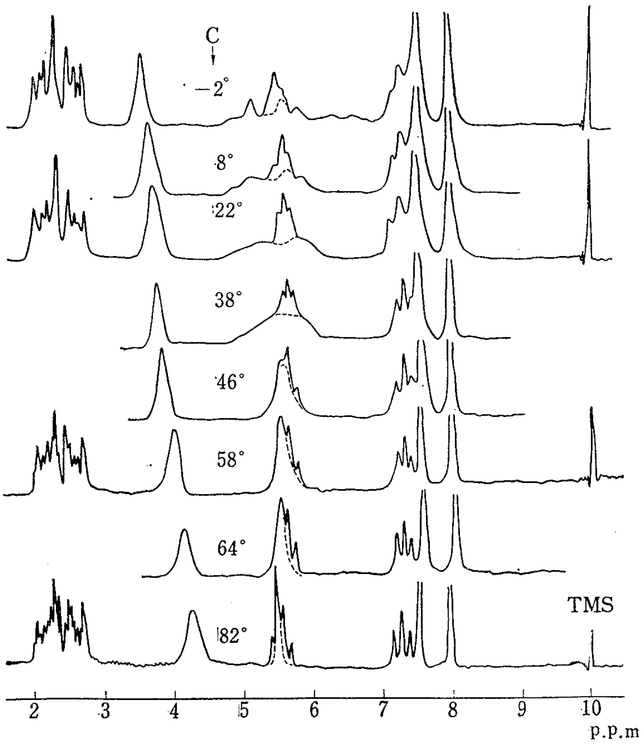

Fig. 1. The Proton Magnetic Resonance Spectra of O-Benzoylthiamine Disulfide in Deuteriochloroform Solution of the Various Temperatures

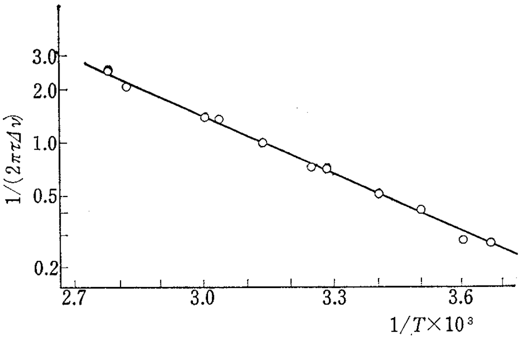

Fig. 2. Temperature Dependence of the Methylene Signal Patterns of O-Benzoylthiamine Disulfide in Deuteriochloroform Solution

Table I. The Differences in Chemical Shifts (τ-p.p.m.) of Various Vitamin B_1

	Substitution of the oxyethyl group					Cyclizing of thiazole ring		
	-O-CO-Ph		-O-Ⓟ					
Protons	OBT	BTDS	TDP	TDMP	BTMP	T	OBT	TDP
$-CH_3$	−0.05	+0.02	−0.02	−0.02	−0.02	−0.36	−0.51	−0.44
$-CH_2-N$	+0.02	+0.03	+0.05	−0.05	+0.02	−1.00	−1.01	−0.9
$-CH_2-$	−0.27	−0.2	−0.18	−0.18	−0.18	−0.6	−0.67	−0.6
$-CH_2-O$	−1.15	−0.66	−0.8	−0.5	−0.36	+0.24	−0.25	±0
Pyrimidine CH	±0	+0.33	+0.12	+0.07	+0.06	−0.02	−0.35	−0.03
Pyrimidine CH_3	−0.03	+0.22	−0.05	±0	+0.31	−0.03	−0.28	−0.08
Thiazole or N-formyl CH	−0.1	±0	±0	+0.05	+0.02			
Reference compds.	T	TDS	T	TDS	TDS	TDS	BTDS	TDMP

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1) E. Grunwald, A. Loewenstein, S. Meiboom : J. Chem. Phys., **27**, 630 (1957).
2) H. S. Gutowsky, C. H. Holm : *Ibid.*, **25**, 1228 (1956).

TABLE II. The Temperature Dependence on the Half Widths (c.p.s.) for the Absorption of Methylene attached to Pyrimidine

temp. (°C) / Sample	20	40	60
BTDS	18.0	9.0	5.0
TDMP	12.0	6.5	3.0
TDS	10.0	5.5	2.5
BTMP	8.5	5.0	2.0
TDP	5.5	3.0	2.0
OBT	5.0	2.5	2.0
MT	4.0	2.0	2.0
ST	3.5	2.5	2.0
T	3.0	2.0	2.0

The temperature dependence observed on the methylene protons attached to pyrimidine ring of disulfide type thiamines would be explained as the hydrogen bonding between N-formyl group and 2-amino group of pyrimidine ring, and as the largeness of the molecular by the disulfide bond.

The author is grateful to Dr. M. Fujisawa and Dr. N. Sugimoto to this Laboratory for their helpful advice and encouragement in this work. He is also indebted to Dr. T. Fujita for the gift of the purified specimens.

Summary

Proton magnetic resonance spectra of nine Vitamin B_1 analogues were measured. The spectral differences between thiazole and disulfide type thiamine were observed on the proton chemical shifts and the temperature dependence of the methylene signals attached to pyrimidine.

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58. **Genshun Sunagawa and Hideo Nakao**: Studies on Seven-membered Ring Compounds. XIII.[*1] Reaction of 2-Bromo-7-methoxytropone with Active Methylene Compounds.

(*Research Laboratories, Sankyo Co., Ltd.*[*2])

It was previously reported[1~4] that the tropone derivatives having a halogen or methoxyl group in the 2-position react with active methylene compounds to yield azulene, 1-azaazulene, 1-oxaazulene or coumarin derivatives. For example, the reaction[1] of ethyl cyanoacetate with 2-chlorotropone (I) affords diethyl 2-amino-1,3-azulenedicarboxylate, ethyl 1-cyano-2-hydroxy-3-azulenecarboxylate and coumarin derivative, and

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[*2] 1-2-58 Hiromachi, Shinagawa-ku, Tokyo (砂川玄俊, 中尾英雄).

1) T. Nozoe, S. Seto, S. Matsumura, T. Asao: Proc. Japan Acad., **32**, 339 (1956).

2) T. Nozoe, S. Seto, S. Nozoe: *Ibid.*, **32**, 472 (1956).

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4) S. Seto, S. Nozoe: Proc. Japan Acad., **32**, 765 (1956).